IMPROVED METHOD OF TREATING TOBACCO TO REDUCE NITROSAMINE CONTENT, AND PRODUCTS PRODUCED THEREBY

CROSS-REFERENCE TO RELATED APPLICATIONS

This application is based on U.S. Provisional Application No. 60/100,372, filed September 15, 1998, and is a continuation-in-part of U.S. Application No. 08/998,043, filed December 23, 1997, which in turn is a continuation-in-part of U.S. Application No. 08/879,905, filed June 20, 1997, which in turn is a continuation-in-part of 08/757,104, filed December 2, 1996 and now U.S. Patent No. 5,803,081 issued to Jonnie R. Williams on September 8, 1998. U.S. Provisional Application No. 60/100,372, U.S. Application Nos. 08/998,043 and 08/879,905, and U.S. Patent No. 5,803,081 are all incorporated herein by reference in their entirety.

FIELD OF THE INVENTION

The present invention relates to an improved method of treating tobacco to reduce the content of, or to prevent the formation of, harmful nitrosamines, which are normally found in tobacco. The present invention also relates to tobacco products having low nitrosamine content.

BACKGROUND OF THE INVENTION

Prior attempts to reduce tar and harmful carcinogenic nitrosamines primarily have included the use of filters in smoking tobacco. In addition, attempts have been made to use additives to block the effects of harmful carcinogens in tobacco. These efforts have failed to reduce the oncologic morbidity associated with tobacco use. It is known that fresh-cut,

green tobacco has virtually no nitrosamine carcinogens. See, e.g., Wiernik et al, "Effect of Air-Curing on the Chemical Composition of Tobacco," Recent Advances in Tobacco Science, Vol. 21, pp. 39 et seq., Symposium Proceedings 49th Meeting Tobacco Chemists' Research Conference, Sept. 24-27, 1995, Lexington, Kentucky (hereinafter "Wiernik et al."). On the other hand, cured tobacco products obtained according to conventional methods are known to contain a number of nitrosamines, including the harmful carcinogens N'-nitrosonornicotine (NNN) and 4-(N-nitrosomethylamino)-1-(3-pyridyl)-1-butanone (NNK). It is widely accepted that such nitrosamines are formed post-harvest, during the conventional curing process, as described further herein. Unfortunately, fresh-cut green tobacco is unsuitable for smoking or other consumption.

It is believed that tobacco-specific nitrosamines (TSNAs) are formed primarily during the curing process. While not wishing to be bound by theory, it is believed that the amount of tobacco-specific nitrosamine (TSNA) in cured tobacco leaf is dependent on the accumulation of nitrites, which accumulate during the death of the plant cell and are formed during curing by the reduction of nitrates under conditions approaching an anaerobic (oxygen deficient) environment. It is believed that the reduction of nitrates to nitrites occur by the action of the micro flora on the surface of the leaf under anaerobic conditions, and it is also believed that this reduction is particularly pronounced under certain conditions (e.g., humid conditions). Furthermore, during the curing process, the tobacco leaf emits carbon dioxide, which can further dilute oxygen levels in the environment.

Once the nitrites are formed, these compounds are believed to combine with various tobacco alkaloids, including pyridine-containing compounds, to form carcinogenic nitrosamines.

tobacco is first air-cured and, when the lamina is yellow or brownish, the tobacco is heated to 65°C for two days in order to cure the stem. An analysis of tobacco produced in this manner showed that both the tobacco-specific nitrosamine (TSNA) and the nitrite contents were low, i.e., in the range of 0.6-2.1 micrograms per gram and less than 10 micrograms per gram, respectively. Wiernik et al theorized that these results were explainable due to the rapid heating which does not allow further bacterial growth. Wiernik et al also noted that tobacco-specific nitrosamine (TSNA) and nitrite contents of 0.2 microgram per gram and less than 15 micrograms per gram, respectively, were obtained for tobacco subjected to air-curing in Poland.

In practice, tobacco leaves are generally cured according to one of three methods. First, in some countries, such as China, a variation of the flue curing process (described below) is still being used on a commercial scale to cure tobacco leaves. Specifically, this variation of the flue curing process features the use of a heat exchanger and involves the burning of fuel and the passing of heated air through flue pipes in a curing barn. Accordingly, in this older version of the curing process, primarily radiant heat emanating from the flue pipes is used to cure the tobacco leaves. While a relatively low flow of air does pass through the curing barn, this process utilizes primarily radiant heat emanating from the flue pipes to cure the tobacco leaves within the barn. In addition, this process does not appreciate, and does not provide for, controlling the conditions within the barn to achieve prevention or reduction of TSNAs. This technique has been largely replaced in the United States by a different flue-curing process.

For more than twenty years, the heat exchanger method described above has been supplanted in the U.S. with a more economical version which features the use of a propane

Accordingly, one object of the present invention is to substantially eliminate or reduce the content of nitrosamines in tobacco intended for smoking or consumption by other means.

Another object of the present invention is to reduce the carcinogenic potential of tobacco products, including cigarettes, cigars, chewing tobacco, snuff and tobacco-containing gum and lozenges.

Still another object of the present invention is to substantially eliminate or significantly reduce the amount of tobacco-specific nitrosamines, including N'-nitrosonornicotine (NNN), 4-(N-nitrosomethylamino)-1-(3-pyridyl)-1-butanone (NNK), N'-nitrosoanatabine (NAT) and N'-nitrosoanabasine (NAB), in such tobacco products.

Another object of the present invention is to treat uncured tobacco at an appropriate time post-harvest so as to arrest the curing process without adversely affecting the tobacco's suitability for human consumption.

Another object of the present invention is to reduce the content of tobacco-specific nitrosamines by treating uncured tobacco in a controlled environment.

Yet another object of the present invention is to reduce the content of tobacco-specific nitrosamines, particularly NNN and NNK, and metabolites thereof in humans who smoke, consume or otherwise ingest tobacco in some form, by providing a tobacco product suitable for human consumption, which product contains a substantially reduced quantity of tobacco-specific nitrosamines, thereby lowering the carcinogenic potential of such product. The tobacco product may be a cigarette, cigar, chewing tobacco or a tobacco-containing gum or lozenge.

Yet another object is to provide a novel curing barn (or curing apparatus) which is

The present invention also seeks to subject tobacco leaves to the controlled environment to prevent normal accumulation of at least one tobacco-specific nitrosamine, such as N'-nitrosonornicotine, 4-(N-nitrosomethylamino)-1-(3-pyridyl)-1-butanone, N'-nitrosoanatabine and N'-nitrosoanabasine.

In another embodiment, the process of the invention further comprises treating the tobacco leaves, while in a state susceptible to having the content of at least one TSNA prevented or reduced, to microwave energy or other forms of high energy treatment.

The present invention in its broadest forms also encompasses a tobacco product comprising non-green tobacco suitable for human consumption and having a lower content of at least one tobacco-specific nitrosamine than conventionally cured tobacco.

In another embodiment, the present invention relates to a novel curing barn which is capable of providing a controlled environment in which the formation of tobacco-specific nitrosamines can be prevented or reduced.

DETAILED DESCRIPTION OF THE INVENTION

For purposes of the invention, the phrase "controlling the conditions" means determining and selecting an appropriate humidity, rate of temperature change, temperature, time of treatment of the tobacco, airflow, CO level, CO_2 level, O_2 level, and arrangement of the tobacco leaves to prevent or reduce the formation of at least one TSNA. For a given set of ambient conditions, it may be necessary to adjust, within the curing apparatus or barn, one or more of these parameters. For example, it is possible to prevent or reduce the formation of TSNAs by simply setting a high airflow through the curing apparatus or barn. In other situations, it is possible to produce the tobacco products of the present invention

with low airflow, provided that other parameters such as humidity, temperature, etc. are appropriately selected.

In this disclosure, tobacco that has been "conventionally cured" is tobacco that has been air-cured or flue-cured, without the controlled conditions described herein, according to conventional methods commonly and commercially used in the U.S.

Further, the term "green tobacco" means tobacco that is substantially uncured and is particularly inclusive of freshly harvested tobacco.

In flue curing processes that utilize a heat exchanger capable of providing relatively low airflow through the curing barn, I have discovered that it is possible to somewhat reduce the TSNA levels by not venting combustive exhaust gases into the curing apparatus or barn. The preferred aspects of the present invention are premised on the discovery that other parameters, as identified above (e.g., airflow), can be adjusted to ensure the prevention or reduction of at least one TSNA regardless of the ambient conditions. Thus, even under the most extreme conditions (i.e., conditions that enhance the formation of TSNAs), it is possible to achieve the prevention or reduction of at least one TSNA.

It has been said that the practice of tobacco curing is more of an art than a science, because curing conditions during any given cure must be adjusted to take into account such factors as varietal differences, differences in leaves harvested from various stalk positions, differences among curing barns in terms of where they are used, and environmental variations during a single season or over multiple seasons, especially in terms of weather fluctuations during air-curing. For example, the practice of flue curing is empirical to a certain degree, and is optimally carried out by individuals who have accumulated experience in this art over a significant period of time. See, e.g., Peele et al, "Chemical and

least one nitrosamine, wherein said controlled environment is provided by controlling at least one of humidity, rate of temperature change, temperature, airflow, CO level, CO_2 level, O_2 level, and arrangement of the tobacco leaves. This treatment of the tobacco essentially arrests the natural formation of TSNAs, and provides a dried, golden yellow leaf suitable for human consumption. If TSNAs have already begun to substantially accumulate, typically toward the end of the yellowing phase, the treatment according to the present invention effectively arrests the natural TSNA formation cycle, thus preventing any further substantial formation of TSNA. When yellow or yellowing tobacco is treated in this fashion at the most optimal time in the curing cycle, the resulting tobacco product has TSNA levels essentially approximating those of freshly harvested green tobacco, while maintaining its flavor and taste. In addition, the nicotine content of the tobacco product according to the present invention remains unchanged, or is substantially unchanged, by the treatment according to the present invention. Accordingly, the tobacco product of the present invention has relatively low contents of TSNAs, and yet the user of the tobacco product can experience the same sensations that are obtainable from using conventional tobacco products.

As discussed above, it is believed that tobacco-specific TSNAs are formed primarily during the curing process. Specifically, it is believed that the amount of TSNAs in cured tobacco leaf is dependent on the accumulation of nitrites, which are formed during the curing process by reduction of nitrates to nitrites under conditions approaching an anaerobic (i.e., oxygen deficient) environment. The nitrites accumulate during the death of the plant cell. Experimental evidence suggests that the nitrites are formed by the micro flora on the surface of the leaf under conditions approaching an anaerobic environment. If, for example,

ensure the prevention or reduction of TSNAs.

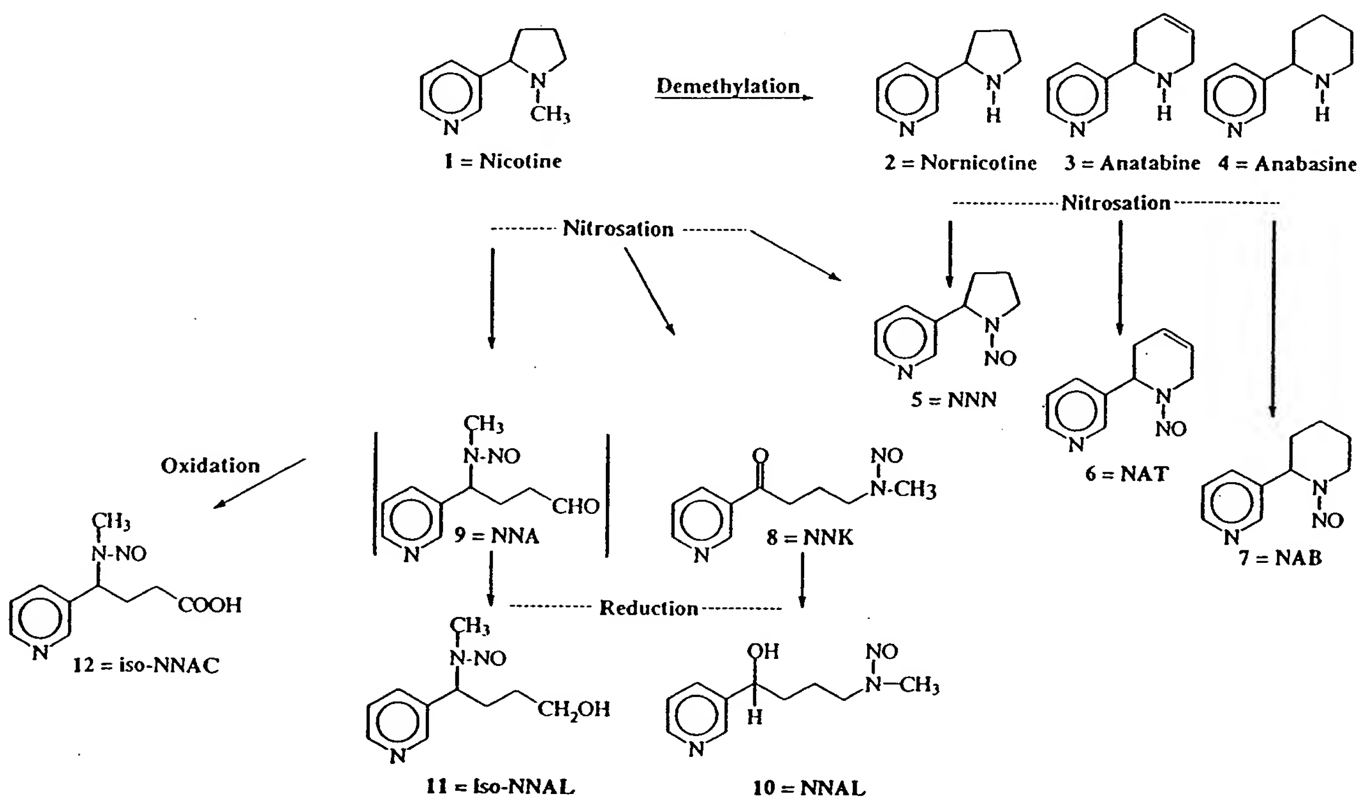
With one particular variety of Virginia flue tobacco on which testing has been carried out as described herein, freshly harvested green tobacco is placed in a barn for about 24-48 hours at about 100-110°F until the leaves turn more or less completely yellow. The yellow tobacco has a reduced moisture content, i.e., from about 90 weight % when green, versus about 70-40 weight % when yellow. At this stage, the yellow tobacco contains essentially no known carcinogens, and the TSNA content is essentially the same as in the fresh-cut green tobacco. This Virginia flue tobacco typically remains in the yellow stage for about 6-7 days. At the end of curing, Virginia flue tobacco typically has a moisture content of about 11 to about 15 weight percent. The conversion of the tobacco during the curing process results in formation and substantial accumulation of nitrosamines, and an increased microbial content. The exact mechanism by which tobacco-specific nitrosamines are formed is not clear, but is believed to be enhanced by microbial activity, involving microbial nitrate reductases in the generation of nitrite during the curing process.

As previously mentioned, tobacco-specific nitrosamines are believed to be formed upon reaction of amines with nitrite-derived nitrosating species, such as NO_2, N_2O_3 and N_2O_4 under acidic or anaerobic conditions. Wiernik et al discuss the postulated formation of TSNAs at pp. 43-45, the discussion being incorporated herein by reference; a brief synopsis is set forth below.

Tobacco leaves contain an abundance of amines in the form of amino acids, proteins, and alkaloids. The tertiary amine nicotine (referenced as (1) in the diagram below) is the major alkaloid in tobacco, while other nicotine-type alkaloids are the secondary amines nornicotine (2), anatabine (3) and anabasine (4). Tobacco also generally contains up

to 5% of nitrate and traces of nitrite.

Nitrosation of nornicotine (2), anatabine (3), and anabasine (4) gives the corresponding nitrosamines: N'-nitrosonornicotine (NNN, 5), N'-nitrosoanatabine (NAT, 6), and N'-nitrosoanabasine (NAB, 7). Nitrosation of nicotine (1) in aqueous solution affords a mixture of 4-(N-nitrosomethylamino)-1-(3-pyridyl)-1-butanone (NNK, 8) (NNN, 5) and 4-(N-nitrosomethylamino)-4-(3-pyridyl)-1-butanal (NNA, 9). Less commonly encountered TSNAs include NNAL (4-N-nitrosomethylamino)-1-(3-pyridyl)-1-butanol, 10), iso-NNAL (4-N-nitrosomethylamino)-4-(3-pyridyl)-1-butanol, 11) and iso-NNAC (4-(N-nitrosomethylamino)-4-(3-pyridyl)-butanoic acid, 12). The formation of these TSNAs from the corresponding tobacco alkaloids is shown schematically below, using the designations 1-12 above (reproduced from Wiernik et al, supra, p. 44, and incorporated herein by reference):



incorporated herein by reference, the preferred aspects of the microwaving or other high energy treatment are described below.

The process of this invention may further comprise a microwaving process for reducing the amount of or preventing formation of nitrosamines in a harvested tobacco plant, which microwaving process comprises

subjecting at least a portion of the plant to microwave radiation, while said portion is uncured and in a state susceptible to having the amount of nitrosamines reduced or formation of nitrosamines arrested, for a sufficient time to reduce the amount of or substantially prevent formation of at least one nitrosamine.

It is preferred that in this aspect of the process of the invention, the step of subjecting to microwave radiation is carried out on a tobacco leaf or portion thereof after onset of yellowing in the leaf and prior to substantial accumulation of tobacco-specific nitrosamines in the leaf. It is also preferred that in this aspect of the process of the invention, the step of subjecting to microwave radiation is carried out prior to substantial loss of the leaf's cellular integrity. Using microwave energy eliminates the potential for activation of the microbes that cause TSNAs in tobacco, particularly in tobacco that has been rehydrated.

The term "microwave radiation" as used herein refers to electromagnetic energy in the form of microwaves having a frequency and wavelength typically characterized as falling within the microwave domain. The term "microwave" generally refers to that portion of the electromagnetic spectrum which lies between the far-infrared region and the conventional radiofrequency spectrum. The range of microwaves extends from a wavelength of approximately 1 millimeter and frequency of about 300,000 MHz to

wavelength of 30 centimeters and frequency of slightly less than about 1,000 MHz. The present invention preferably utilizes high power applications of microwaves, typically at the lower end of this frequency range. Within this preferred frequency range, there is a fundamental difference between a heating process by microwaves and by a classical way, such as by infrared (for example, in cooking): due to a greater penetration, microwaves generally heat quickly to a depth several centimeters while heating by infrared is much more superficial. In the United States, commercial microwave apparatuses, such as kitchen microwave ovens, are available at standard frequencies of approximately 915 MHz and 2450 MHz, respectively. These frequencies are standard industrial bands. In Europe, microwave frequencies of 2450 and 896 MHz are commonly employed. Under properly balanced conditions, however, microwaves of other frequencies and wavelengths would be useful to achieve the objects and advantages of the present invention.

Microwave energy can be generated at a variety of power levels, depending on the desired application. Microwaves are typically produced by magnatrons, at power levels of 600-1000 watts for conventional kitchen-level microwave apparatuses (commonly at about 800 watts), but commercial units are capable of generating power up to several hundred kilowatts, generally by addition of modular sources of about 1 kilowatt. A magnatron can generate either pulsed or continuous waves of suitably high frequency.

The applicator (or oven) is a necessary link between the microwave power generator and the material to be heated. For purposes of the present invention, any desired applicator can be used, so long as it is adapted to permit the tobacco plant parts to be effectively subjected to the radiation. The applicator should be matched to the microwave generator to optimize power transmission, and should avoid leakage of energy towards the outside.

dryer. Thus, it is within the present invention to operate the process of the present invention in a convective heating air oven or a clothes dryer, although these apparatuses are not within the scope of the curing apparatus or barns as defined in the appended claims.

In another embodiment, the present invention relates to a tobacco product comprising cured non-green or yellow tobacco suitable for human consumption and having a content of at least one tobacco-specific nitrosamine selected from N'-nitrosonornicotine (NNN), 4-(N-nitrosomethylamino)-1-(3-pyridyl)-1-butanone (NNK), N'-nitrosoanatabine (NAT) and N'-nitrosoanabasine (NAB) which is less than about 50% by weight of the content of said at least one tobacco-specific nitrosamine in conventionally cured tobacco, more preferably less than about 75% by weight, most preferably less than about 95% by weight, without the use of organic solvent extraction.

Thus, it is possible to reduce the TSNA content by about 97% or more by practicing the present invention, even down to "food safe" TSNA levels.

For example, the NNN level of the tobacco product according to the present invention is typically less than about 0.05 μg/g, the combined NAT and NAB level is typically less than about 0.10 μg/g, and the NNK level is typically less than about 0.05 μg/g. Further, the combined TSNA level is typically less than about 0.16 μg/g, even as low as less than about 0.009 μg/g.

Thus, in yet another aspect of the present invention, the tobacco product according to the present invention comprises cured non-green or yellow tobacco having a NNN content less than about 0.05 μg/g.

In a further aspect, the tobacco product of the present invention comprises cured non-green or yellow tobacco having a combined NAT and NAB content of less than about

Example 6

Yellow tobacco leaf in the inner portion of a curing barn was subjected to a flow of air for 7 days according to the present invention. The results are tabulated in Table 3.

Comparative Example 5

Yellow tobacco leaf cured in a curing barn according to a conventional curing process was tested for TSNA levels. The results are shown in Table 3.

Table 3

EXAMPLE NO.	μg/g NNN	μg/g NAT + NAB	μg/g NNK	μg/g TSNA
Ex. 5	0.03±.02	0.06	0.05	0.14±.02
Ex. 6	0.04±.01	0.08±.02	0.04	0.15±.01
Comp. Ex. 5	0.41±.04	1.16±.13	1.56±.21	3.14±.36

As is apparent from Table 3, the curing process according to the present invention provided unexpectedly lower levels of TSNA as compared to a conventional curing process.

Example 7

This example illustrates the advantageous effects obtainable by practicing the present invention even under the most severe environmental conditions. Throughout all phases of the curing, combustion exhaust gases were not allowed to flow into the barn.